



## Product Information

**MONOCLONAL ANTI-HUMAN TUMOR  
NECROSIS FACTOR SOLUBLE RECEPTOR I (sTNF RI)  
CLONE 16803.1  
Purified Mouse Immunoglobulin**

Product Number **T 1690**

### Product Description

Monoclonal Anti-Human Soluble Tumor Necrosis Factor Receptor I (sTNF RI) (mouse IgG1 isotype) is derived from the 16803.1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with recombinant human sTNF-RI, expressed in *E. coli*. The antibody is purified from ascites fluid using protein A chromatography.

Monoclonal Anti-Human sTNF-RI may be used to neutralize the biological activity mediated by sTNF RI. By immunoblotting and ELISA, the antibody shows no cross reactivity with recombinant human sTNF-RII, recombinant mouse sTNF-RI and sTNF-RII.

Monoclonal Anti-Human sTNF-RI may be used for neutralization of the biological activity mediated by sTNF RI and the detection of sTNF RI by immunoblotting and ELISA.

TNF RI (CD120a) is a 55 kDa transmembrane glycoprotein that is expressed by virtually all nucleated mammalian cells.<sup>1-3</sup> Among the numerous cells known to express TNF RI are hepatocytes,<sup>4</sup> monocytes and neutrophils,<sup>5</sup> cardiac muscle cells,<sup>6</sup> endothelial cells,<sup>7</sup> and CD34<sup>+</sup> hematopoietic progenitors.<sup>8</sup> Both TNF- $\alpha$  and TNF- $\beta$  bind to TNF RI. Soluble TNF- $\alpha$  binds with a Kda in the range of 20-60 pM,<sup>9,10</sup> while TNF- $\beta$  binds with a Kda equal to 650 pM.<sup>9</sup> TNF RI relative to TNF RII seems to be the more physiologically-relevant receptor, whereas TNF-R2 appears to play a direct role in only a limited number of TNF responses.<sup>11,12</sup> Soluble TNF RI, which blocks TNF- $\alpha$  activity, has been identified in both urine and blood (1-3 ng/mL).<sup>4,13,14</sup> Serum levels of sTNF receptors increase dramatically in certain pathological situations. Two soluble forms have been identified and are believed to be generated by proteolytic cleavage.<sup>3,15-17</sup>

Human TNF RI has 64% amino acid sequence identity (70% in the extracellular region) with mouse TNF-R1, and human TNF RI binds human and mouse TNF- $\alpha$  with equal affinity.<sup>18,19</sup>

The extracellular region has four cysteine-rich motifs, the first of which is suggested to be required for binding.<sup>9</sup> The intracellular portion of TNF R1 contains a "death domain" of about 70 amino acids that is required for the signaling of apoptosis and NF- $\kappa$ B activation.<sup>20,21</sup> TNF binds to the extracellular domain of TNF R1 and induces receptor trimerization.<sup>22</sup> Then, the aggregated death domain of TNF R1 recruits the adapter protein TRADD.<sup>21</sup> TRADD, in turn, recruits FADD, TRAF2 and RIP to form the TNF R1 signaling complex and activate signaling cascades leading to apoptosis,<sup>23,24</sup> JNK/SAPK activation,<sup>23,25</sup> and NF- $\kappa$ B activation<sup>26,27</sup> respectively. However, TNF R1 self-associates and signals independently of ligand when overexpressed. This apparent paradox may be explained by silencer of death domains (SODD), a widely expressed approximately 60 kDa protein that was found to be associated with the death domain of TNF-R1.<sup>28</sup>

### Reagents

The product is supplied lyophilized from a 0.2  $\mu$ m filtered solution in phosphate buffered saline. Endotoxin level is < 10 ng per mg antibody as determined by the LAL method.

### Preparation Instructions

To one vial of lyophilized powder, add 1 ml of 0.2  $\mu$ m-filtered PBS to produce a 0.5 mg/ml stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

### Storage/Stability

Store at -20 °C.

Reconstituted product may be stored at 2-8 °C for at least one month. For prolonged storage, freeze in working aliquots at -20 °C. Avoid repeated freezing and thawing.

### Procedure

Anti-Human sTNF RI is tested for its ability to neutralize the biological effect of sTNF-RI but not sTNF RII in cytolytic assay using mouse L929 cells, in the presence of TNF- $\alpha$ .<sup>29</sup> The ND<sub>50</sub> of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of the soluble receptor activity on a responsive cell line, when the soluble receptor is present at a concentration just high enough to elicit a maximum response.

### Product Profile

For neutralization, a working concentration of 3 – 6  $\mu$ g/ml of Monoclonal Anti- sTNF RI will block 50% of the bioactivity due to 0.3  $\mu$ g/ml recombinant human sTNF R1, in the presence of 0.25 ng/ml TNF- $\alpha$  in a crystal violet cytolytic assay using mouse L929 cells.

For Indirect Immunoblotting, a working concentration of 1-2  $\mu$ g/ml is determined using recombinant human sTNF RI at 20 ng/lane under non-reducing conditions and 300 ng/lane under reducing conditions.

For Indirect ELISA, a working concentration of 0.5 - 1  $\mu$ g/ml is determined to detect recombinant human sTNF RI to a limit of 0.78 ng/well.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

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